

Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions

M. Grawehr¹, B. Sener¹, T. Waltimo² & M. Zehnder¹

¹Division of Endodontology, Department of Preventive Dentistry, Cariology, and Periodontology, University of Zürich Center for Dental Medicine, Zürich, Switzerland; and ²Department of Cariology and Endodontics, Institute of Dentistry, University of Turku, Turku, Finland

Abstract

Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. *International Endodontic Journal*, **36**, 411–415, 2003.

Aim To evaluate interactions of ethylenediamine tetraacetic acid (EDTA) with sodium hypochlorite (NaOCl).

Methodology Solutions consisting of 8.5% EDTA and 0.5% NaOCl were compared to a 1 : 1 (w/w) mixture of 17% EDTA and 1% NaOCl for their calcium-chelating, tissue-dissolving, and antimicrobial properties. Amounts of available chlorine were determined in the EDTA/NaOCl solutions with an iodine/thiosulphate titration method. Calcium chelation capacity was titrated with a pure calcium solution using a murexide indicator. Weight loss of porcine palatal mucosal specimens incubated in the test solutions was measured over time. Antimicrobial potential of pure solutions and the combination was recorded using an agar diffusion test in plates incubated with *Enterococcus faecalis* or *Candida albicans*.

Results The presence of hypochlorite had little effect on the calcium-chelating ability or on the antimicrobial potential of EDTA. Available chlorine content decreased to 0.06% in the combined EDTA–NaOCl solution compared to 0.50% in an equivalent NaOCl mixture with deionized water. The EDTA–NaOCl solution did not dissolve more tissue than an equivalent pure EDTA solution at any time (ANOVA, $P > 0.05$).

Conclusions Ethylenediamine tetraacetic acid retained its calcium-complexing ability when mixed with NaOCl, but EDTA caused NaOCl to lose its tissue-dissolving capacity and virtually no free chlorine was detected in the combinations. Clinically, this suggests that EDTA and NaOCl should be used separately. In an alternating irrigating regimen, copious amounts of NaOCl should be administered to wash out remnants of the EDTA.

Keywords: antimicrobial effect, calcium chelation, EDTA, sodium hypochlorite, tissue dissolution.

Received 23 September 2002; accepted 11 February 2003

Introduction

The goal of the cleaning and shaping regimen in endodontic therapy is to maximally reduce microbial load and necrotic tissue remnants in the root canal system. Mechanical instrumentation leaves around 40–50% of the root canal walls untouched (Peters *et al.* 2001). Irrigating solutions are therefore required in conjunction with mechanical preparation for optimal results (Baker

et al. 1975). Two frequently used irrigants in endodontics are aqueous solutions of sodium hypochlorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA).

A 0.5% NaOCl solution buffered with sodium bicarbonate was originally described for the cleansing of open wounds in World War I (Dakin 1915). In today's endodontic practice, NaOCl solutions are used at concentrations ranging from 0.5 to 5.25%. Such 'chlorinated soda' irrigants were introduced to the dental literature in 1936 (Walker 1936). Sodium hypochlorite has good tissue-dissolving capacity and dentine-disinfecting potential (Zehnder *et al.* 2002). However, it has only a limited effect on the dissolution of smear layer and dentine. It has therefore been recommended to use demineralizing agents as adjuvants in root canal treatment

Correspondence: Dr Matthias Zehnder, Division of Endodontics, Department of Preventive Dentistry, Cariology, and Periodontology, University of Zürich Center for Dental Medicine, Plattenstrasse 11, CH 8028 Zürich, Switzerland (Tel.: +41 1632 8610; fax: +41 1634 4308; e-mail: matthias.zehnder@zzmk.unizh.ch).

(Nygaard Östby 1957). Opened dentinal tubules may allow the hypochlorite solution to better penetrate dentine, leading to cleaner root canals (Goldman *et al.* 1982). An alternating irrigation regimen of sodium hypochlorite and EDTA is more efficient than NaOCl alone in reducing the bacterial load in the root canal system (Byström & Sundqvist 1985).

The sodium salts of EDTA are noncolloidal organic chelating agents, which have the ability to form nonionic chelates with metallic ions such as Ca^{2+} . EDTA solutions are most active at a pH between 7 and 8, and are thus believed to be more tissue-friendly than other acid-based demineralizing agents (Nikiforuk & Sreebny 1953).

Little is known of chemical interactions between EDTA and NaOCl and the resulting biological consequences (Baumgartner & Ibay 1987, Saquy *et al.* 1994). The goal of the present study was to evaluate some effects of mixing EDTA and NaOCl solutions. Combined EDTA–NaOCl and equivalent pure solutions were studied for their calcium-chelating potential, free available chlorine content, tissue-dissolution capacity and antimicrobial effectiveness.

Materials and methods

Chemical interactions of NaOCl and EDTA

The 17% EDTA (w/v) solution used in the present study was obtained by a pharmacist upon prescription; the NaOCl solutions were prepared from a pure 10% stock solution. The effect of the presence of sodium hypochlorite on the chelating ability of EDTA was tested with a calcium titration method, using murexide as an indicator (Schulze & Simon 1989). In a 50 mL test tube, 0.3 g of the 17% EDTA solution was mixed with 20 mL of deionized water. The solution was rendered alkaline by the addition of 5 mL of a 2 : 1 (w/w) 0.1 M glycine/0.1 M NaOH buffer (Sørensen 1909). Subsequently, murexide was added, and the solution was titrated with a 500 p.p.m. Ca^{2+} solution ($1.2492 \text{ g CaCO}_3 \text{ L}^{-1} \text{ H}_2\text{O}$) till its colour changed from purple to red, indicating the presence of nonchelated Ca^{2+} ions. A freshly prepared 9 : 1 (w/w) mixture of 17% EDTA and 5% NaOCl was subjected to the same procedure. The calcium-chelating potential of both the pure EDTA and the EDTA/NaOCl solutions was compared by calculating the amount of chelated calcium per mole of EDTA.

To test the chemical effect of EDTA on NaOCl, free available chlorine content ($\text{HOCl} + \text{OCl}^-$) of a 1 : 1 (w/w) 1% NaOCl/ H_2O mixture was compared to a freshly prepared 1 : 1 (w/w) 1% NaOCl/17% EDTA solution. A

standard iodine/thiosulphate titration method was used for this purpose (Vogel 1962).

Tissue dissolution assay

Full-thickness palatal mucosa was dissected from four pigs within 30 min after slaughtering, as described previously (Zehnder *et al.* 2002). The pigs were raised and slaughtered for food production, according to the Swiss standards for animal welfare. The study protocol did not, in any way, influence the premortal fate of the animals or the slaughtering process. Therefore, this investigation was not classified as an animal study, and the institutional ethics committee did not have any objections to the protocol. Tissue samples were shock-frozen on dry ice and kept at -27°C in plastic bags to prevent freeze-drying until further usage.

Frozen tissue specimens of defined surface and similar weight of approximately 80 mg were obtained with a round punch of 5 mm inner diameter applied at the crest of the rugae. Subsequently, tissue specimens were thawed in 30 mL isotonic saline at room temperature for 30 min. Eight specimens each were incubated at 32°C , the average intracanal temperature (Cunningham & Balekjian 1980), in separate test tubes each containing 30 mL of the following solution: 8.5% EDTA, 0.5% NaOCl, a 1 : 1 (w/w) mixture of 17% EDTA with 1% NaOCl, or saline. At the start of the incubation and after 15, 30, 60, 90 and 120 min exposure to the solution, samples were washed in saline, blotted dry and weighed using a precision balance in an air-tight container (AT 261, Mettler, Greifensee, Switzerland). Results are expressed as the percentage of the original tissue weight.

Agar diffusion test

Facultative bacteria (*Enterococcus faecalis* ATCC 29212) and yeasts (*Candida albicans* OMZ 110) were maintained separately in fluid universal medium (FUM; Gmür & Guggenheim 1983) supplemented with 5% foetal bovine serum. The microbiota were chosen because they are the most frequently isolated facultatives and yeasts from root canals of failed endodontic treatments (Dahlén *et al.* 2000, Waltimo *et al.* 1997). The optical density of the suspensions containing the microbes was measured at a wavelength of 550 nm and adjusted by dilution with FUM to an optical density of 1.0. Fifteen microlitres of this agar was inoculated in Petri dishes with 5 mL of the broth containing one of the microorganisms. After the agar had set, round cavities (5 mm in diameter) were punched out. These cavities were filled with 40 μL of

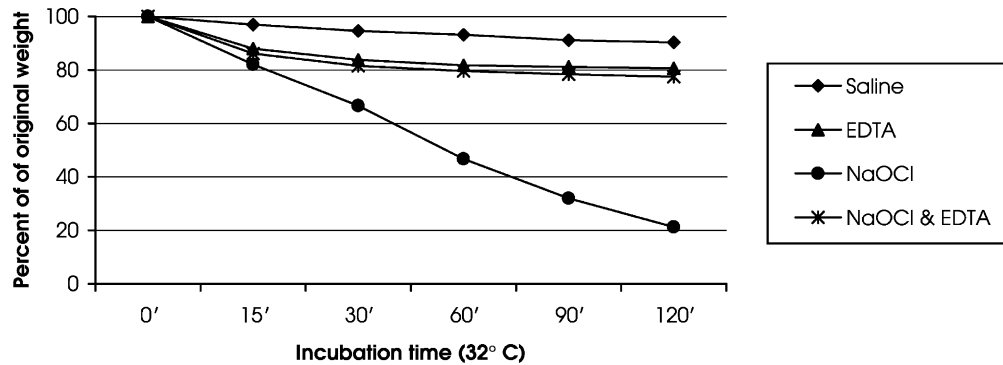


Figure 1 Necrotic porcine palatal tissue was exposed to an 8.5% EDTA solution, a 0.5% NaOCl solution, a 1 : 1 (w/w) combination of 17% EDTA with 1% NaOCl, or saline. Per cent of remaining tissue weight is plotted as a function of time (min).

the solutions described in the tissue dissolution assay. After 24 h incubation at 37 °C in ambient air, zones of inhibition were measured with a caliper. All experiments were performed in triplicates. Results are expressed as millimetres of zones of inhibition.

Data presentation and analysis

Data pertaining to the tissue dissolution and the agar diffusion assays are presented as means and SDs. Box plots of data obtained in the tissue dissolution assay displayed even distribution, and parametric statistics were therefore applied (Altman *et al.* 1983). Tissue dissolution values obtained at different time-points were compared using one-way analysis of variance (ANOVA) followed by an unpaired *t*-test. Bonferroni adjustment was applied for multiple post hoc testing. The level for rejection of the null hypothesis was set at $P < 0.05$.

Results

Chemical interactions of NaOCl and EDTA

In a solution containing both EDTA and NaOCl, the presence of hypochlorite had little effect on the calcium-

chelating ability of EDTA. In a pure solution, EDTA chelated $0.82 \text{ mol Ca}^{2+} \text{ mol}^{-1}$, compared to $0.77 \text{ mol Ca}^{2+} \text{ mol}^{-1}$ EDTA in the EDTA/NaOCl mixture. In contrast, EDTA had a substantial effect on the hypochlorite. In a freshly prepared 1 : 1 mixture of 17% EDTA and 1% NaOCl, available chlorine content was 0.06%, compared to 0.50% in a 1 : 1 mixture of the same NaOCl solution with deionized water.

Tissue dissolution assay

The EDTA and NaOCl solutions, as well as the EDTA/NaOCl mixture, dissolved significantly more tissue than saline at all times (Fig. 1, ANOVA, $P < 0.05$). The pure 0.5% hypochlorite solution was substantially more efficient than all other solutions ($P < 0.05$), and left only $21.2 \pm 1.6\%$ of the original tissue weight after 120 min of incubation. The 1 : 1 17% EDTA/1% NaOCl mixture never dissolved a significantly different amount of tissue than the 8.5% EDTA solution ($P > 0.05$).

Agar diffusion test

Zones of inhibition against *C. albicans* and *E. faecalis* were of the same width whether a pure 8.5% EDTA solution or the 17% EDTA/1% hypochlorite combination was used (Table 1). EDTA had a substantial inhibitory effect on the growth of *C. albicans*, with a mean inhibition zone of $9.8 \pm 0.3 \text{ mm}$ compared to $1.5 \pm 0.0 \text{ mm}$ observed with a pure 0.5% hypochlorite solution.

Discussion

Little information is available on the direct interaction of EDTA and sodium hypochlorite in the endodontic literature. This would appear to be the first report on the tissue

Table 1 Zones of inhibition (mm) against test organisms (agar diffusion)

	<i>Enterococcus faecalis</i> ATCC 29212	<i>Candida albicans</i> OMZ 110
Saline	0	0
NaOCl 0.5%	1.3 ± 0.3^a	1.5 ± 0.0
EDTA 8.5%	3.0 ± 1.0	9.8 ± 0.3
EDTA 17% and NaOCl 1% (1 : 1)	3.0 ± 1.3	9.8 ± 0.3

^aMean value $\pm$ SD ($n = 3$).

dissolution and antimicrobial potential of a direct EDTA/NaOCl combination.

The current study confirms an earlier report on the retained chelating potential of EDTA solutions in the presence of hypochlorite (Saquy *et al.* 1994). In that investigation, a Ni-dimethylglyoxime titration method, flame spectrometry and measurements of dentine microhardness were used to study the influence of hypochlorite on EDTA. Measurements of chlorine gas evaporation in EDTA/NaOCl mixtures have suggested an interaction of sodium hypochlorite with the acidic hydrogens of EDTA, eventually leading to chlorine gas evaporation and a reduction of available chlorine in solution (Baumgartner & Ibay 1987). The stoichiometry of that reaction has been published in the latter article. In the present study, available chlorine content in a combined EDTA–hypochlorite solution was measured. This more direct method demonstrated an almost complete absence of free chlorine (OCl^- and HOCl), thus confirming the calculations of Baumgartner & Ibay (1987).

The recent finding that the tissue-dissolving potential of hypochlorite solutions is mainly a function of free available chlorine (Zehnder *et al.* 2002) was corroborated by the current study. A simple yet reliable weighing method was chosen to determine tissue dissolution because irrigating solutions interfere with total protein assays and with hydroxyproline determination (Koskinen *et al.* 1980). An agar diffusion test was selected to study the antimicrobial potential of test solutions. With that method, EDTA had a substantial antifungal effect, which has already been reported by other investigators using a similar method (Sen *et al.* 2000). EDTA solution was also found to be more efficient against *E. faecalis* than a 0.5% NaOCl solution, both in the current and in a previous study using an agar diffusion model (Siqueira Junior *et al.* 1998). However, in the root canal system, EDTA probably binds to dental hard tissues and may be inactivated. This may explain why EDTA has little disinfecting capacity in tubules of infected dentine blocks (Heling & Chandler 1998; Ørstavik & Haapasalo 1990). Nevertheless, EDTA appears to have antimicrobial efficacy in necrotic soft tissues of the root canal system (Yoshida *et al.* 1995). To study the latter hypothesis, more data on the influence of organic material, blood, tissue fluids and dentine on EDTA are required. When EDTA was preincubated with dentine powder, microbial growth inhibition was strongly reduced (data not shown). The antiseptic EDTA effect is a consequence of the metallic ion-chelating ability of EDTA (Vaara 1992). Based on the calcium-chelating experiment presented

here, it was therefore surmised that NaOCl did not hamper the antimicrobial effect of EDTA.

The exact mechanism of microbial killing by hypochlorite solutions is not well elucidated. However, it is most likely that this action is a function of the available chlorine content, as is the tissue-dissolution capacity. In an aqueous environment, NaOCl dissociates to OCl^-/HOCl (hypochloric acid, at pH 12 mostly OCl^- ; Bloomfield & Miles 1979). Both molecules are strong oxidizing agents. Although not directly shown in the present study, it may be assumed that EDTA inhibits the antimicrobial efficacy of hypochlorite solutions by elimination of free chlorine.

Conclusions

- 1 Under the conditions of the present study, an EDTA solution maintained its calcium-chelating ability and its antimicrobial effectiveness when combined with NaOCl.
- 2 NaOCl lost available chlorine and therefore its tissue-dissolving effectiveness when EDTA was added.
- 3 It may be clinically advisable to copiously rinse root canals with NaOCl after each EDTA irrigating step to wash out the remaining EDTA and guarantee good effectiveness of the hypochlorite solution.

Acknowledgements

The authors would like to thank Martin Gander for pre-culturing the microbiota used in the present study.

References

- Altman DG, Gore SM, Gardner MJ, Pocock SJ (1983) Statistical guidelines for contributors to medical journals. *British Medical Journal (Clinical Research Edition)* **286**, 1489–93.
- Baker NA, Eleazer PD, Averbach RE, Seltzer S (1975) Scanning electron microscopic study of the efficacy of various irrigating solutions. *Journal of Endodontics* **1**, 127–35.
- Baumgartner JC, Ibay AC (1987) The chemical reactions of irrigants used for root canal debridement. *Journal of Endodontics* **13**, 47–51.
- Bloomfield SF, Miles GA (1979) The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. *Journal of Applied Bacteriology* **46**, 65–73.
- Byström A, Sundqvist G (1985) The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *International Endodontic Journal* **18**, 35–40.
- Cunningham WT, Balekjian AY (1980) Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. *Oral Surgery, Oral Medicine Oral Pathology Oral Radiology and Endodontics* **49**, 175–7.

- Dahlén G, Samuelsson W, Molander A, Reit C (2000) Identification and antimicrobial susceptibility of enterococci isolated from the root canal. *Oral Microbiology and Immunology* **15**, 309–12.
- Dakin HD (1915) On the use of certain antiseptic substances in treatment of infected wounds. *British Medical Journal* **2**, 318–20.
- Gmür R, Guggenheim B (1983) Antigenic heterogeneity of *Bacteroides intermedius* as recognized by monoclonal antibodies. *Infection and Immunity* **42**, 459–70.
- Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS (1982) The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study. Part 2. *Journal of Endodontics* **8**, 487–92.
- Heling I, Chandler NP (1998) Antimicrobial effect of irrigant combinations within dentinal tubules. *International Endodontic Journal* **31**, 8–14.
- Koskinen KP, Stenvall H, Uitto VJ (1980) Dissolution of bovine pulp tissue by endodontic solutions. *Scandinavian Journal of Dental Research* **88**, 406–11.
- Nikiforuk G, Sreebny L (1953) Demineralization of hard tissues by organic chelating agents at neutral pH. *Journal of Dental Research* **32**, 859–67.
- Nygaard Östby B (1957) Chelation in root canal therapy. *Odontologisk Tidskrift* **65**, 3–11.
- Ørstavik D, Haapasalo M (1990) Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endodontics and Dental Traumatology* **6**, 142–9.
- Peters OA, Laib A, Gohring TN, Barbakow F (2001) Changes in root canal geometry after preparation assessed by high-resolution computed tomography. *Journal of Endodontics* **27**, 1–6.
- Saquet PC, Maia Campos G, Sousa Neto MD, Guimaraes LF, Pecora JD (1994) Evaluation of chelating action of EDTA in association with Dakin's solution. *Brazilian Dental Journal* **5**, 65–70.
- Schulze G, Simon J (1989) *Massanalyse*. Berlin: Walter de Gruyter, 214–6.
- Sen BH, Akdeniz BG, Denizci AA (2000) The effect of ethylenediamine-tetraacetic acid on *Candida albicans*. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **90**, 651–5.
- Siqueira Junior JF, Batista MMD, Fraga RC, de Uzeda M (1998) Antibacterial effects of endodontic irrigants on black-pigmented Gram-negative anaerobes and facultative bacteria. *Journal of Endodontics* **24**, 414–6.
- Sørensen SPL (1909) Enzymstudien. II. Mitteilung über die Messung und die Bedeutung der Wasserstoffionenkonzentration bei enzymatischen Prozessen. *Biochemische Zeitschrift* **21**, 131–200.
- Vaara M (1992) Agents that increase the permeability of the outer membrane. *Microbiological Reviews* **56**, 395–411.
- Vogel AI (1962) *A Textbook of Quantitative Inorganic Analysis*, 3rd edn. London: Longmans, 363–5.
- Walker A (1936) A definite and dependable therapy for pulpless teeth. *Journal of the American Dental Association* **23**, 1418–25.
- Waltimo TM, Sirén EK, Torkko HL, Olsen I, Haapasalo MP (1997) Fungi in therapy-resistant apical periodontitis. *International Endodontic Journal* **30**, 96–101.
- Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I (1995) Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. *Journal of Endodontics* **21**, 592–3.
- Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T (2002) Tissue dissolving capacity and antimicrobial effect of buffered and unbuffered hypochlorite solutions. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **94**, 756–62.